IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of	:	Knipe, et al.
Serial No.	:	08/278,601
Filed	:	July 21, 1994
For	:	Herpesvirus Replication Defective Mutants
Group	:	1817
Examiner	:	Caputa

Assistant Commissioner of Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R.§1.608(b)

I, Stephen A. Rice, declare:

1. That prior to September 25, 1990 I worked in the Laboratory of Dr. David Knipe, one of the named inventors of the above-captioned application. My curriculum vitae is attached hereto as Appendix A.

2. That the following is a factual description of experiments performed by me in the United States prior to September 25, 1990.

3. That I was requested to perform these experiments by Dr. David Knipe.

4. That Appendix B attached hereto are true copies, with dates deleted, of laboratory notebook pages written by me in conjunction with the performance of the experiments performed by me in the United States before September 25, 1990, and that the notebook pages of Appendix B accurately report the following experiments that were performed by me.

5. That the experiments I performed in the United States prior to September 25, 1990 were as follows:

An ICP27 gene nonsense codon insertion mutant herpesvirus n504R was propagated and titrated on V-27 cells. V-27 cells can produce the mutant herpesvirus because they contain an integrated copy of the ICP27 gene, thus they complement the growth of HSV-1 ICP27 mutants and serve as hosts for the isolation of ICP27 mutants. A replication defective mutant such as n504R will not however replicate on normal cells such as Vero cells which are routinely used for growth of HSV-1, since they lack an ICP27 gene.

V-27 cells were infected with an n504R mutant herpesvirus. After harvesting the propagated herpesvirus from the cells, the n504R mutant herpesvirus was aliquoted and frozen at –70 and designated "M28 n504R". A sample of the virus was tested for its ability to replicate by measuring plaque formation in cells infected with the mutant herpesvirus. The mutant herpesvirus n504R was tested for plaque formation on V-27 cells and on Vero cells. The results showed that the n504 mutant herpesvirus failed to produce plaques on the Vero cells at the lowest dilution (10^{-3}) that could be read (lower dilutions killed the monolayer). At that same low dilution the V-27 cell monolayer had too many plaques to count. Distinct and measurable plaques were formed on the V-27 cells at much higher dilutions tested in the V-27 cells (10^{-7}). A virus titer of 4×10^{8} was calculated. These results confirmed that n504R is a replication defective ICP27 mutant herpesvirus.

6. That on information and belief, an aliquot of this n504R stock was delivered to the laboratory of Dr. Robert Finberg, one of the named inventors of the above-captioned application, prior to September 25, 1990.

7. That the following correlates the above-described experiment to the notebook pages provided in Appendix B:

A. The notebook page records the making of a stock of n504.a mutant herpesvirus. This is stated on the second line of the page: "For each stock:.....n504R".

B. Cells that had been infected and incubated were harvested, frozen and thawed twice and sonicated. The harvested virus was aliquoted, tubes labeled and frozen. This is stated on the first notebook page at the third line:

"Shake off.
Into 2 200 ml bottle.
10', 1.5K
Resuspend in 20 ml 10 ml supe + 10 ml milk.
Freeze @ -70
Thaw 2x
Sonicate 3x30" @ setting 4.
Aliquot to 40 vials.
"M28 n504R".

C. Three days later, the virus was tested at a series of dilutions (10^{-1} to10^{-8}) for plaque formation on V27 and Vero cells. There was no plaque formation in the Vero cells and positive plaque formation in the V-27 cells. The titer of the mutant virus was calculated to be 4 x 10^8. This is stated on the notebook second page:

"	-1	-2	-3	-4	-5	-6	-7	-8
n504R-V27			>>	>>	>>	>>	31	2
n504R-Vero	k	k	0	0	0	0		

k= killed monolayer

Titers for muts	V27:	Vero
n504R	4 x 10^8	< 1 x 10^{3}"

8. That I hereby declare that all statements made herein are true, and all statements made on information and belief are believed to be true, and further that all statements were made with the knowledge that any willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Date: Aug. 31, 1998

Stephen A. Rice

APPENDIX A

CURRICULUM VITAE

Name: Stephen A. Rice

Address: Department of Microbiology
University of Minnesota Medical School
1435 Mayo Memorial Building
420 Delaware St.
Minneapolis, MN 55455-0312

Phone: (612) 626-4183
Fax: (612) 626-0623
e-mail: stever@lenti.med.umn.edu

Date of Birth: December 9, 1955

Citizenship: U.S.A. (permanent resident status in Canada)

Education:

1978 B.S.	University of California, Davis (Biochemistry, with honors)
1985 Ph.D.	University of Utah, Salt Lake City, Utah (Cellular, Viral and Molecular Biology). Supervisor: Dr. Daniel Klessig, "A Genetic Analysis of the Adenovirus-specified DNA-binding Protein"

Post-doctoral Training:

1985-1990	Harvard Medical School, Boston, Mass., Department of Microbiology and Molecular Genetics. Supervisor: Dr. David Knipe

Academic Positions:

1990-1996	Assistant Professor, Department of Biochemistry, University of Alberta, Edmonton, Alberta
1996-1998	Associate Professor (with tenure), Department of Biochemistry, University of Alberta
1997-1998	Visiting Scientist, Department of Microbiology, University of Minnesota, Minneapolis; sabbatical research in the laboratory of Dr. Paul Siliciano, Dept. of Biochemistry
1998-2000	Associate Professor (on leave), Department of Biochemistry, University of Alberta
1998-	Senior Research Associate, Department of Microbiology, University of Minnesota, Minneapolis

Awards and Fellowships:

1981-1985	National Institutes of Health Predoctoral Training Award
1985-1988	National Institutes of Health NRSA Postdoctoral Fellowship
1988-1990	Ernst Fellowship
1990	Establishment grant ($220,500 Canadian award to establish laboratory), Alberta Heritage Foundation for Medical Research, (AHFMR)
1990-1995	Heritage Medical Scholar (competitive salary award), AHFMR
1990-1993	Research operating grant ($299,208 Can.), National Cancer Institute of Canada (NCIC)
1990	Terry Fox Equipment Award ($75,000 Can.), NCIC
1993-1994	Research operating grant, one year extension ($38,600 Can.)
1993-1995	Research operarating grant ($16,000 Can.), Cancer Research Society
1994-1997	Research operating grant ($304, 470 Can.), NCIC
1994-1997	Research operating grant, Medical Research Council of Canada (MRC) (declined)
1995-2000	Heritage Senior Medical Scholar (competitive salary award), AHFMR
1997-1998	Research operating grant ($124,030 Can.), NCIC
1997-2000	Research operating grant, MRC (declined)
1997-1998	Research operating grant ($20,500 Can.), Alberta Cancer Board

Curent Research Support:

1998-2003	"Molecular characerization of herpes simplex virus ICP27", NIH: RO1-AI42737-01, $151,697 U.S. direct costs in year one (sponsored by Microbiology Dept., University of Minnesota Medical School).

University Teaching:

1991-1996	Biochemistry 450, "*Molecular Biology of Mammalian Viruses*" (9 lectures/ term; taught in alternating years)
1992-1997	Biochemistry 201/205, "*General Biochemisty*" (18 lectures / term; taught yearly)
1992-1997	Biochemistry 430, "*Biochemistry of Nucleic Acids and Gene Regulation*" (9 lectures/ term; taught in alternating years)

Student and post-doctoral training:

Ph.D. students:

1991-1997	Wendy Mears (studentship award from AHFMR)
1993-present	Scott Bunnell (studentship award from AHFMR)

Post-doctoral fellows:

1997-1998	Wendy Mears
1997-1998	Melissa Long

1997-present Henry Parker

Graduate Student Supervisory Committees:
Have sat on more than twenty University of Alberta M.Sc. and Ph.D Supervisory committees since 1990

Ph.D. Examination Committees, External Examiner:
1994 Randall Berg, University of Calgary
1995 Frank Jones, McMaster University

University Administative Duties:

1991-1994	Medical Sciences Library Committee (Faculty of Medicine)
1992-1997	DNA Core Facility Users Committee (Biochemistry Department)
1994-1997	Faculty Retreat Organizing Committee (Biochemistry Department)

Professional Activities:

1991-present	Reviewer for: National Science and Engineering Research Council of Canada Medical Research Council of Canada Saskatchewan HSURC Alberta Cancer Board *Virology* *Journal of Virology*
1994	Organizing Committee, 19th International Herpesvirus Workshop, Vancouver, B.C., July 30-Aug. 4, 1994
1996-1997	Scientific Officer, NCIC Grants Review Panel F (Virology, Gene Expression, and Structural Biology)

Memberships:

1990-present	American Association for the Advancement of Science
1990-present	American Society of Microbiology

Community Activities:

1994	Spoke on cancer research to the Alberta organizers of the 1994 Terry Fox Runs, Edmonton
1995	Spoke on cancer research at opening ceremonies of 1995 Edmonton Terry Fox Run, Edmonton; media interviews
1996	Team Organizer (University of Alberta Cancer Research Group), 1996 Terry Fox Run

Invited Seminars:

University of Calgary/ Alberta Molecular Oncology Retreat, Banff, Alberta, June 1992.
University of Alberta, Anatomy and Cell Biology Department, June 1992.
University of Alberta, Genetics Department, February 1993.

Bio-Mega/ Boehringer Ingelheim Research, Inc., Biochemistry Dept., Laval, Quebec, May 1994.
University of Calgary, Department of Medical Biochemistry, December 1994.
University of Alberta Faculty of Medicine, J.B. Collip Club, March 1995.
SUNY Health Science Center, Department of Microbiology and Immunology, Syracuse N.Y., May 1995.
University of Minnesota, Department of Veterinary PathoBiology, St. Paul, Minnesota, May 1995.
McMaster University, Dept. of Pathology, Hamilton, Ontario, November 1995.
Uniformed Services University of the Health Sciences, Dept. of Microbiology and Immunology, Bethesda, Maryland, January 1996.
Southwest Foundation for Biomedical Research, Dept. of Virology and Immunology, San Antonio, Texas, February 1996.
Mt. Sinai School of Medicine, Microbiology Dept., New York, New York, March 1996.
University of Minnesota, Dept. of Microbiology, January 1997.
Medical College of Wisconsin, Microbiology Dept., April 1997.

Peer-reviewed Publications:

Rice, S.A. and Klessig, D.F. The function(s) provided by the adenovirus-specified DNA-binding protein required for late viral gene expression is independent of the protein's role in viral DNA replication. *J. Virol.* **49**:35-45 (1984).

Brough, D.E., Rice, S.A., Sell, S. and Klessig, D.F. Restricted changes in the adenovirus-specified DNA binding protein that lead to extended host range or temperature-sensitive phenotypes. *J. Virol.* **55**:206-212 (1985).

Rice, S.A. and D.F. Klessig. Isolation and analysis of adenovirus type 5 mutants containing deletions in the gene encoding the DNA-binding protein. *J. Virol.* **56**:767-778 (1985).

Klessig, D.F., Rice, S.A., Cleghon, V., Brough, D.E., Williams, J.F. and Voelkerding, K. Studies on the adenovirus DNA-binding protein. *In:* Botchan, M., T. Grodzicker, P.A. Sharp (eds), *Cancer Cells* : Vol. 4 - DNA tumor viruses: Control of gene expression and replication. New York: Cold Spring Harbor Laboratory. pp. 485-496 (1986).

Rice, S.A., Klessig, D.F. and Williams, J. Multiple effects of the 72-kDa, adenovirus-specified DNA-binding protein on the efficiency of cellular transformation. *Virology* **156**: 366-376 (1987).

Knipe, D.M., Senechek, D., Rice, S.A. and Smith, J.L. Stages in the nuclear association of the herpes simplex virus transcriptional activator protein ICP4. *J. Virol.* **61**: 276-284 (1987).

Nabel, G.J., Rice, S.A., Knipe, D.M. and Baltimore, D. Alternative mechanisms for activation of human immunodeficiency virus enhancer in T cells. *Science* **239**: 1299-1302 (1988).

Rice, S.A. and Knipe, D.M. Gene-specific transactivation by the herpes simplex virus alpha protein ICP27. *J. Virol.* **62**: 3814-3823 (1988).

Rice, S.A., Su, L. and Knipe, D.M. Herpes simplex virus alpha protein ICP27 possesses separable positive and negative regulatory activities. *J. Virol.* **63**: 3399-3407 (1989).

Rice, S.A. and Knipe, D.M. Genetic evidence for two distinct transactivation functions of the herpes simplex virus alpha protein ICP27. *J. Virol.* **64**: 1704-1715 (1990).

Rice, S.A., Lam, V, and Knipe, D.M. The acidic amino-terminal region of the herpes simplex virus type type 1 alpha protein ICP27 is required for an essential lytic function. *J. Virol.* **67**: 1778-1787 (1993).

Rice, S.A, Long, M.C., Lam, V. and Spencer, C.A. RNA polymerase II is aberrantly phosphorylated and localized to viral replication compartments following HSV infection. *J. Virol.* **68**: 988-1001 (1994).

Rice, S.A. and Lam, V. Amino acid substitution mutations in the herpes simplex virus ICP27 protein define an essential gene regulation function. *J. Virol.* **68**: 823-833 (1994).

Winkler, C.A., Rice, S.A. and Stamminger, T. UL69 of human cytomegalovirus, an open reading frame with homology to ICP27 of herpes simplex virus, encodes a transactivator of gene expression. *J. Virol.* **68**: 3943-3954. (1994)

Upton, C.A., Schiff, L., Rice, S.A., Dowdeswell, T. , Yang, X. and McFadden, G. A novel poxvirus protein binds zinc through a ring finger motif and localizes in virus factories. *J. Virol.* **68**: 4186-4195. (1994)

Mears, W., Lam, V., and S. Rice. Identification of nuclear and nucleolar localization signals in the herpes simplex virus regulatory protein ICP27. *J. Virol.* **69**: 935-947. (1995)

Rice, S., Long, M., Lam, V., Schaffer, P. and C. Spencer. Herpes simplex virus immediate-early function ICP22 is required for modification of host RNA polymerase II and establishment of the normal viral transcription program. *J. Virol.* **69**: 5550-5599. (1995)

Lees-Miller, S., M. Long, A. Kilvert, V. Lam, S. Rice, and C. Spencer. Attenuation of DNA-dependent protein kinase activity and its catalytic subunit by the HSV-1 transactivator, ICP0. *J. Virol.*, **70**: 7471-7477. (1996)

Mears, W. and S. Rice. The RGG box motif of the herpes simplex virus ICP27 protein mediates an RNA-binding activity and determines *in vivo* methylation. *J. Virol.* **70**:7445-7453. (1996)

Spencer, C., M. Dahmus, and S.A. Rice. Repression of host RNA polymerase II transcription by herpes simplex virus type 1. *J. Virol.* **71**: 2031-2040. (1997)

Mears, W., and S. Rice. The herpes simplex virus regulatory protein ICP27 shuttles between the nucleus and cytoplasm. *Virology* **242**:128-137. (1998).

Long, M., V. Leong, P. Schaffer, C. Spencer, and S. Rice. The UL13 virion protein kinase and ICP22 regulatory protein are both required for herpes simplex virus-induced modification of RNA polymerase II. Manuscript in preparation.

Bunnell, S. and S. Rice. Isolation of viable herpes simplex virus mutants expressing a truncated, frame-shifted form of the ICP27 regulatory protein. Manuscript in preparation.

~PM. Cells look ready to harvest, but let go till the morrow.

For each stock: 406R & 504R

Shake off
Into 2 200 ml bottle.
10, 1.5 k

Resuspend in 20 ml 10 ml supe + 10 ml milk.

Freeze @ -70

Thaw 2x

Sonicate 3 x 30" @ setting 4.

Aliquot to ~40 vials. "M28 n406R".
"M28 n504R".

Freeze @ -70°.

Titer on V27, Vero's

V27's just confluent
Vero's nice

	V27	Vero
n406R	-3 -4 -5 -6 -7 -8	-1 -2 -3 -4 -5 -6
n504R	-3 -4 -5 -6 -7 -8	-1 -2 -3 -4 -5 -6
KOS	-3 -4 -5 -6 -7 -8	-3 -4 -5 -6 -7 -8

0.2 ml 1.0 ml etc.

6 well trays
1 ml/well

V27's are light;
add, i.e. overlay w/
med + 10% serum + 1g fraction in
0.16 ml 1g fraction in 100 ml
use 75 ml med is 0.1 ml in use 2x)

	-1	-2	-3	-4	-5	-6	-7	-8
n406R - V27			>>	>>	>>	>>	31	2
n504R - V27			>>	>>	>>	>>	32	3
kos - V27			>>	>>	>>	>>	54	11
n406R - Vero	k	k	ø	ø	ø	ø		
n504R - Vero	k	k	ø	ø	ø	ø		
kos - Vero			>>	>>	>>	>>	105	23

k = killed monkeys

Titers for mnts V27:

		Vero
n406R	3×10^8	$< 1 \times 10^3$
n504R	4×10^8	$< 1 \times 10^3$